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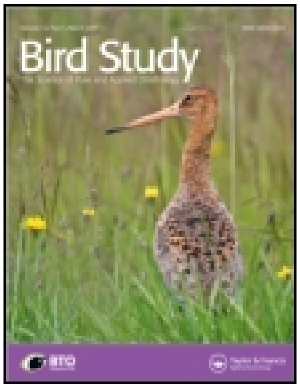

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SHORT REPORT

Egg hatchability in high Arctic breeding wader species Charadriiformes is not affected by determining incubation stage using the egg flotation technique

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Capsule By following the fate of both floated and non-floated eggs from individual clutches of four Arctic wader (shorebird) species, we show that the use of the flotation method for determining incubation stage of eggs (which involves both the submersion and handling of eggs) does not negatively affect hatchability.

Egg floating (van Paassen *et al.* 1984) is known to be a reliable method for assessing the developmental stage of eggs during incubation (e.g. Westerskov 1950, van Paassen *et al.* 1984, Liebezeit *et al.* 2007, Ackerman & Eagles-Smith 2010). Because of its reliability and ease of use, the method is employed regularly in field studies of birds. However, only limited information is available on the possible negative effects on hatchability. Although Schreiber (1970) called for further research into the subject, the notion that flotation has no negative effect has often been stated (e.g. Devney *et al.* 2009, Mabee *et al.* 2006), although actual results were not reported. The study by Alberico (1995) was the first rigorous attempt to test if the use of egg flotation represents any significant negative effect on egg hatchability. However, Alberico (1995) followed the fates of entire clutches rather than individual eggs within a clutch, which may have masked a negative effect of flotation on egg hatchability. In the present study, we tested the potential negative effect of the flotation method (including handling of the egg) on egg hatchability by examining hatchability of floated versus non-floated eggs within individual clutches. Specifically, we used wader (shorebird) nesting data collected at Zackenberg Research Station as part of the ongoing monitoring programme BioBasis (see Schmidt *et al.* 2010), where egg flotation is used to determine nest initiation and hatching dates of local breeding waders (cf. Meltofte *et al.* 2007). The dominant breeding wader species at Zackenberg are Sanderling *Calidris alba*, Dunlin *Calidris alpina* and Ruddy Turnstone *Arenaria interpres*, with lower densities of breeding Common Ringed Plover *Charadrius hiaticula*, Red Knot *Calidris canutus* and Red-necked Phalarope *Phalaropus lobatus* (Meltofte 2006). In the BioBasis programme, eggs of waders are checked at the nest to determine incubation stage by floating two eggs of the clutch in a 0.5-litre transparent container 2/3-filled with water at a temperature of approximately 25°C from a vacuum flask. This water temperature was chosen as it lies within the thermal tolerance for short cold temperature exposure on eggs (16–41°C; Webb 1987). Standard measures, such as angle between the egg axis and the bottom of the vessel, or the extent of the egg that is above water, and the angle towards the water surface, were recorded. Eggs were floated for a few seconds only and each egg was floated only once. A typical nest visit took between 5 and 10 minutes. Nests were not visited on days with inclement weather. Most nests were visited only a few times, i.e. at discovery (where flotation takes place), approximately 3 days prior to expected hatching, and at hatching. If the clutch of hatchlings was not in the nest cup at our final visit, hatching was verified by the presence of egg shell

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fragments embedded in the nest material, while nest predation was indicated by various factors, such as pierced eggs shells, egg yolk, smell of Arctic Fox *Vulpes alopex*, etc. (see Mabee *et al.* 2006).

During the breeding seasons of 2007, 2008 and 2009, we tested whether the hatchability of floated eggs differed from those of non-floated eggs. Waders typically lay clutches of four eggs (Hale 1980). We floated two randomly selected eggs and left the other two untouched in each clutch. After carefully drying the floated eggs with paper tissue, we cautiously placed a pencil mark on the egg shell just before putting the eggs back in the nest. The marks enabled us to see whether any unhatched eggs had previously been floated, thus creating both a treated group and a control group within each clutch.

We tested the effect of floating on egg hatchability using two approaches. First, we tested hatchability of floated versus non-floated eggs across the years 2007–2009 using the traditional Fisher's Exact Test (H_0: no difference in hatchability between floated and non-floated eggs). Secondly, to remedy the potential impact of pseudoreplication of eggs within nests, we also tested the effect of species and treatment (floated or not floated), along with their interaction, on egg hatchability, using a mixed logistic model with nest as random factor. Data on wader eggs that suffered predation ($n = 383$), suffered damage ($n = 2$), were trampled by musk oxen ($n = 3$), or from nests with unknown fates ($n = 28$) were omitted, leaving a total of 266 eggs from 71 nests for analysis, i.e. 123 Sanderling eggs, 89 Dunlin eggs, 42 Ruddy Turnstone eggs and 12 Red Knot eggs. Eggs that did not hatch were examined for embryos, and the developmental stage of each embryo was estimated. Also, if possible, the most likely cause of hatching failure was noted for each egg (e.g. inclement weather, flooding, etc.). During all three breeding seasons 2007–2009, the handling and floating procedures remained the same. During the work, the ethical guidelines promoted by the Association for the Study of Animal Behaviour (ASAB 2004) were followed.

The results of Fisher's tests of hatchability of individual eggs in relation to floating clearly showed that egg floatation, which included both the handling and actual submersion of eggs, did not influence the egg hatchability of any of the four wader species examined ($P > 0.245$ for all four wader species; Table 1). These results were supported by the results from the mixed model, where neither floating ($P = 0.832$), species ($P = 0.111$), nor their interaction ($P = 0.810$) affected egg hatchability. During the three breeding seasons, a total of 27 eggs were found abandoned and unhatched. Of these eggs,

Table 1. Impact of the flotation method on the hatchability of eggs of four species of high arctic breeding waders. Data collected at the Zackenberg Research Station, Greenland, over three seasons, 2007–2009. *P* values refer to Fisher's exact test, with 1 degree of freedom. There are no significant differences between eggs which were floated and those which were not.

		Hatching		
Species	Floating	No	Yes	*P*
Dunlin	No	2	45	0.496
	Yes	0	42	
Red Knot	No	0	6	1.000
	Yes	0	6	
Sanderling	No	10	60	0.245
	Yes	12	41	
Ruddy Turnstone	No	0	22	1.000
	Yes	0	20	

Table 2. The developmental stages of embryos in unhatched floated and unhatched non-floated eggs from two species of high arctic breeding waders. Data collected at the Zackenberg Research Station, Greenland, over three seasons, 2007–2009.

		Embryo development			
Species	Egg floated	No visible embryo	Small embryo	Large embryo	No data
Dunlin	No	1			1
	Yes				
Sanderling	No	9		1	
	Yes	12			

two were probably abandoned due to severe weather events, and one egg due to human disturbance (one egg not fully hatched at nest visit during hatching). The remaining eggs (12 floated and 12 non-floated eggs, from a total of 14 clutches) were abandoned for no obvious reason and thus did not hatch (Table 2).

Based on our detailed examination of 266 wader eggs, the results from the present study showed that the flotation method (i.e. both egg handling and submersion in water) is indeed a viable solution for determining incubation stage, and poses no significant threat to hatchability. Even when examining the developmental stage of unhatched eggs, it is evident that hatching failure occurs in both floated and non-floated eggs, and that hatching failure is not associated with application of the flotation method. One explanation for the observed hatching failure is infertility, which has been reported to be the most important factor for hatching failure (Cabezas-Daz & Virgós 2007). In addition to infertility, exposure to temperature stress during female off-duty time during the incubation period has been mentioned as a prime factor in egg mortality (Castilla *et al.* 2009), although incubating adults adjust their incubation schedules to ambient temperatures to minimize unnecessary cooling of their clutches (Reneerkens *et al.* 2011). We conducted our study in high Arctic Greenland, where ambient temperatures are well below the thermal tolerance for short exposure on eggs (Webb 1987), but we still did not observe any detrimental effects of flotation. However, hyperthermia (when body temperature rises above the required temperature for normal metabolism), may be more harmful to embryos than hypothermia (Webb 1987). Willemsen *et al.* (2010) showed that broiler chicken embryos exposed to low incubation temperatures showed delayed hatching process, while similar embryos exposed to high incubation temperatures exhibited more severe responses, such as reduced embryo growth and altered levels of carbohydrate and lipid metabolism. Embryonic development and growth in the embryos exposed to cold were similar to those of a control group, indicating that cold is less of a threat to embryonic development than is heat. In waders, incubating birds seem able to compensate for delayed embryo growth due to cold exposure by raising the egg temperature (Reneerkens *et al.* 2011). Avoiding excessive heating of eggs, and hence detrimental effects, in warm environments may be much more difficult (Webb 1987). Evaporation of water costs energy (Grant 1982) and eggs will therefore loose heat after submersion into water, through the water residue on the egg shell. In cold environments this is a matter of concern, as the heat loss to the cold ambience is likely to be an important factor. Applying the methods the way we have done evidently decreases these threats to an acceptable level.

While we provide strong evidence that egg flotation has no detrimental effect on egg hatchability, we urge researchers to use the method carefully, not least under harsh climatic conditions. Although not specifically tested for in our study, we suggest that when using the flotation method water should have an approximate temperature of 25°C, i.e. within the range of the temperature range suggested by Webb (1987), and egg submersion time should be minimized. Also, we recommend that the time spent at the nest is minimized; this will reduce general disturbance and also reduce the likelihood that olfactory cues, which could attract predators, are left at the nest site.

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